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RESEARCH ARTICLE

STUDY ON EXPLORATION OF EFFECT OF VOLTAGE GATED CALCIUM CHANNEL BLOCKERS ON THE ANTI DEPRESSANT ACTION OF IMIPRAMINE AND ALPRAZOLAM

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ABSTRACT

Background: Imipramine is a tricyclic anti depressant drug. Alprazolam is a benzodiazepine sedative drug, but it has also antidepressive action. Voltage gated calcium channel blockers are well known antihypertensive, anti anginal, anti arrhythmic drugs. **Objective:** In this study I explored the effects of calcium channel antagonists on the antidepressant action of alprazolam and imipramine. **Materials And Methods**: Despair swim test model was used to study the anti depressive effect on the Male Sprague-Dawley rats. Rats were divided into nine groups (n = 6 per group). One group received a single dose of Tween 80 solution, as because it was used as vehicle for all the drugs; two groups each received a single dose of the antidepressant alone (alprazolam or imipramine); two groups each received a single dose of the calcium channel blocker (nifedipine or verapamil); four groups each received a single dose of the calcium channel blocker followed by a single dose of the antidepressant (with same doses used for either in the previous four groups). Drug administration was performed concurrently on the nine groups. **Results:** The anti depressant action of both imipramine and alprazolam was confirmed by this study. . Both verapamil & nifedipine delays the onset of immobility, when administered separately. Verapamil potentiate the antidepressive effect of both imipramine & alprazolam. When nifedipine was combined with imipramine , there was delay in the onset of immobility and was greater than their single use. . Either imipramine or nifedipine produced a delay in the onset of immobility of 75% and 81%, respectively, compared to the control($p< 0.05$) and combining nifedipine with imipramine led to a delay of 73% in the onset of immobility compared to the control ($p< 0.05$). **Conclusion:** Combination of voltage gated calcium channel blocker with imipramine and alprazolam was some positive effect on the antidepressant action.
Key words: Anti depressive action, Sedative, Voltage gated calcium channel, Alprazolam, Imipramine.

INTRODUCTION

Depression and anxiety disorders are the most common mental illnesses, each affecting in excess of 10-15% of the population at some time in their lives. Both anxiety and depressive disorders are amenable to pharmacological treatments that have been developed since the 1950s [1]. Imipramine is tertiary amine tricyclic antidepressive drug. It enhances monoaminergic neurotransmission by inhibiting the synaptic reuptake of both nore-epinephrine and serotonin [2].

Alprazolam is belonging to the benzodiazepines. It has the capacity to promote the binding of the major inhibitory neurotransmitter γ-aminobutyric acid (GABA) to the $GABA_A$ subtype of GABA receptors, which exist as multi-subunit, ligand-gated chloride channels, thereby enhancing the GABA-induced ionic currents through these channels [3, 4].

Voltage-sensitive Ca^{2+} channels (L-type or slow channels) mediate the entry of extracellular Ca^{2+} into smooth muscle and cardiac myocytes and sinoatrial (SA) and atrioventricular (AV) nodal cells in response to electrical depolarization. In both smooth muscle and cardiac myocytes, Ca^{2+} is a trigger for contraction, albeit by different mechanisms. Ca^{2+} channel antagonists, also called Ca^{2+} entry blockers, inhibit Ca^{2+} channel function. In vascular smooth muscle, this leads to relaxation, especially in arterial beds [5]. Verapamil is belonging to the phenylalkylamine compound. Verapamil enhanced the antidepressant action of alprazolam [6], Verapamil as an inhibitor of the CYP 450 3A4 may affect the imipramine and alprazolam action, that are considered as substrates for CYP 450 3A4[7].

Porsolt et al. [8] proposed Despair swim test (DST) as a model to test for antidepressant activity of any substances. It was suggested that mice or rats forced to swim in a restricted space from which they cannot escape are induced to a characteristic behavior of immobility. This behavior reflects a state of despair which can be reduced by several agents, which are therapeutically effective in human depression [9]. The rat DST model has been widely used in screening antidepressants because it is simple and has been reported to be reliable across laboratories. The rat version seems to be more selective (fewer false positives).The rat model is more sensitive than the mouse model because it produces fewer false negatives [10]. The DST is specific enough to discriminate between antidepressants, neuroleptics and anxiolytics [11]. Behavioral despair is mediated by central catecholamines. Drugs that increase central transmission of dopamine or NA decrease immobility, whereas agents having the opposite effect increase immobility. The advantage of the mouse DST model is that it can readily test the possible mechanisms of antidepressant action by using specific agonists/antagonists. By augmenting or blocking antidepressant activity with agonist/antagonist receptor ligands, it is possible to detect which receptor is involved in the antidepressant effect [12].

In this study behavior despair models was used to investigate the effect of the calcium channel blockers, nifedipine and verapamil, on the antidepressant action of

alprazolam and imipramine. These two calcium channel blockers are used in the treatment of physical illnesses that may be concurrent with depression. Understanding the interaction between antidepressants and calcium channel blockers could indicate whether there is a need to modify antidepressant doses when co-administered with calcium channel blockers.

MATERIALS AND METHODS

Ethical Consideration: Prior to the initiation of the study, necessary permission was obtained from the Institutional Animal Ethics Committee. And the maintenance of the animals as well all the procedures of the experiment were as per the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines.

Study duration: The study was conducted during the month of January to March 2006, at the Pharmacology Department of Burdwan Medical College, West Bengal, India.

Study Design and Methodology: 54 Male Sprague-Dawley rats weighing 160–180 g are collected from the institutional animal house. They were kept in standard plastic rat cages and fed with a standard rat food which was in pellet form (which was bought from Hindustan Animal Feeds) and tap water. The rooms were equipped with lighting, conditioning, moisture and heat control. Groups of rats were housed in separate cages. The animals were housed at room temperature (20–25°C) and a 12-h dark/light cycle. The animals were adapted to their new surroundings for three days before the initiation of the experiment.

Because alprazolam is not freely soluble in saline, all the drugs were dissolved in 1% Tween 80 in distilled water. They were injected intraperitoneally. Imipramine was given at 12 mg/kg and alprazolam at 6 mg/kg. The doses of calcium channel blockers were then selected accordingly.

Rats were divided into nine groups (n = 6 per group). One group received a single dose of 6ml/kg of 1% Tween 80; two groups each received a single dose of the antidepressant alone (alprazolam, 6mg/kg; imipramine, 12mg/kg); two groups each received a single dose of the calcium channel blocker (nifedipine, 7mg/kg; verapamil, 12mg/kg); four groups each received a single dose of the calcium channel blocker followed by a single dose of the antidepressant (with same doses used for either in the previous four groups). Drug administration was performed concurrently on the nine groups. For all groups, the time of onset of immobility was measured 60 min after drug administration. In this study I had chosen the Despair swim test (DST) as proposed by Porsolt et al. [8].

Rats were individually forced to swim inside a vertical Plexiglas cylinder (height: 40 cm; diameter: 18 cm, containing 15 cm of water maintained at 25 °C). Rats placed in the cylinders for the first time were initially highly active, vigorously swimming in circles, trying to climb the wall or diving to the bottom. After 2–3 min activity began to subside and to be interspersed with phases of immobility or floating of increasing length. After 5–6 min immobility reached a plateau where the rats remain immobile for approximately 80% of the time. After 15 min in the water the rats were removed and allowed to dry in a heated enclosure (32 °C) before being returned to their home cages. They were again placed in the cylinder 24 h later and the total duration of immobility was measured during a 5 min test. Floating behavior during this 5 min period had been found to be reproducible in different groups of rats. An animal was judged to be immobile whenever it remains floating passively in the water in a slightly hunched but upright position, its nose just above the surface. Test drugs or standard were administered one hour prior to testing.

Statistical analysis: All the collected data were analyzed by using the **S**tatistical **P**ackage for the **S**ocial **S**cience (SPSS**) ver-16** in Windows-7. If the data were normally distributed one way Anova test was applied. And if the data were not normally distributed, groups were compared using Wilcoxon signed rank test. A value of $p < 0.05$ was considered statistically significant.

RESULTS AND ANALYSIS

There was an effect of nifedipine on the onset of immobility: Administration of imipramine, alprazolam, or nifedipine separately produced a significant delay in the onset of immobility compared to the control group. The combined administration of alprazolam and nifedipine produced a significant delay in the onset of immobility compared to either alprazolam treated rats or the control group. The effect of imipramine on the onset of immobility (delay) was potentiated by the administration of nifedipine (**Table 1).**

Table 1: Effects of Nifedipine on the onset of immobility by alprazolam and imipramine using the despair swim test model of depression. The values are expressed as mean ± SD (a): $p \leq 0.05$ compared to control group treated with Tween 80- treated. (b): $p \leq 0.05$ compared to group treated with alprazolam + nifedipine. (c): $p \leq 0.05$ compared to group treated with imipramine + nifedipine.

Treatment (n= 6) Table No. 1	Onset of Immobility (Seconds)
Tween 80	39.09 ± 0.93
Alprazolam (6 mg/ kg)	52.09 ± 1.09 (a, b)
Alprazolam (6mg/kg) & Nifedipine(7mg/kg)	69.15 ± 1.17 (a)
Imipramine (12mg/kg)	79.17 ± 1.08 (a, c)
Imipramine (12mg/kg)&Nifedipine(7mg/kg)	82.5 ± 1.09 (a)
Nifedipine (7mg/kg)	59.08 ± 1.59 (a, b, c)

Effects of verapamil on the onset of immobility: Administration of verapamil, alprazolam or imipramine produced a significant delay in the onset of immobility compared to the control group. Co-administration of

verapamil augmented the effects of imipramine. Similarly, coadministration of verapamil augmented the effects of alprazolam. My findings demonstrate that verapamil significantly delays the onset of immobility produced by alprazolam (Table 2).

Table 2: Effects of verapamil on the onset of immobility produced by alprazolam or imipramine using the despair swim test model of depression. The values are expressed as mean ± SD. (a): p ≤ 0.05 compared to control group treated with Tween 80-treated. (b): p ≤ 0.05 compared to group treated with alprazolam + verapamil. (c): p ≤ 0.05 compared to the group treated with imipramine + verapamil.

Treatment (n= 6) Table No.2	Onset of Immobility (Seconds)
Tween 80	39.09 ± 0.93
Alprazolam (6mg/kg)	52.09 ± 1.09 (a, b)
Alprazolam (6mg/kg)& Verapamil (12 mg/ kg)	80.9 ± 0.89 (a)
Imipramine (12 mg/kg)	79.17 ± 1.08 (a, c)
Imipramine (12 mg/kg)& Verapamil (12 mg/kg)	91.08 ± 1.09 (a)
Verapamil (12mg/kg)	58.01 ± 0.02 (a, b, c)

DISCUSSION

It has been suggested that calcium channel inhibitors may have antidepressant properties, and that calcium may play an important role in affective disorders. Voltage-dependent calcium channel antagonists have been reported to produce antidepressant-like effects in rodents. Interruption of the Ca^{2+}-calmodulin-NOS-guanylyl cyclase subcellular signaling pathway at any point produces antidepressant-like effects [13]. In my study, nifedipine delayed the onset of immobility in the forced swimming maze. This antidepressant action could have been mediated by 5-HT_{1A} activation, whereby nifedipine reduced 5-HT uptake. This would lead to an increase in the cytosolic calcium activity via 5-HT_2 receptors [14]. Serotonin may activate calcium influx through calcium channels by activation of 5- HT receptors, which are insensitive to nifedipine, in neuronal cells. The increase in calcium influx is through 5-HT_3 receptors, the 5- HT_3 receptor being a ligand-gated ion channel activated by the neurotransmitter serotonin. Receptors of this subtype have been localized to several regions of the brain; they appear to be involved in many neuronal functions, and to mediate antidepressant effects [15]. In glial cells, the increase in intracellular calcium is through 5- HT_2 receptors [14]. It has been suggested that the pharmacology of L-type Ca^{2+}-channel blockers overlaps with that of 5-HT_2 receptor antagonists [15]. Nifedipine may produce an antidepressant action through $GABA_A$ activation, which leads to the release of NA that produces an antidepressant effect [16]. This may be GABA acting on second inhibitory interneurons (as in direct and indirect pathways of extrapyramidal systems). Nifedipine may also produce its antidepressant effect by increasing the release of intracellular calcium through $GABA_A$ receptors and NA [16]. The central antidepressant effect of nifedipine may be mediated through an interaction, with novel modulatory sites on $GABA_A$ receptors, that is not through picrotoxin, flumazenil [17].

Verapamil showed an anti depressant like effect as it delayed the onset of immobility in the swimming maze. Studies have shown that verapamil modulates the action of antidepressant drugs that down regulate β adrenergic systems [17]. It has a similar final effect as β blockers, and shares this effect with antidepressant drugs. Working on different types of calcium entry, verapamil blocks the prejunctional α_2 receptors, which leads to an increase in NA release [18]. Verapamil may have direct catecholamine releasing effects, as it interacts with catecholamine storage vesicles in a way that reduces their ability to take up and store catecholamine, and thereby increasing NA release from sympathetic nerves [17]. Verapamil has no effect on NA-induced increase in calcium influx, which means that there are verapamil sensitive and verapamil-insensitive calcium channels [18]. Verapamil enhances ATP response, which is released along with NA from the motor nerves; ATP may indeed be a co-transmitter. Many investigators suggested that ATP induces NA release from sympathetic neurons via its action on a subclass of the nicotinic cholinoceptor**,** because this effect was blocked by nicotinic receptor antagonists [16]. NA produces depolarization by decreasing the membrane permeability to K+ ions. It also increases calcium influx to the cells via calcium channel-activated NA receptors and potential-dependent slow calcium channels activated by NA-induced membrane depolarization [14]. NA stimulates calcium chloride conductance, leading to opening of voltage-gated calcium channels [15].

Imipramine can inhibit presynaptic reuptake of the biogenic amines, serotonin, and NA to produce an antidepressant action [19].Imipramine may produce this antidepressant action through a GABA–ergic mechanism, causing release of catecholamine. Imipramine may increase calcium release from intracellular stores by increasing NA concentrations through inhibiting its uptake by pre-synaptic sites through $GABA_A$ receptor activation. This may lead to increased calcium influx through voltage gated calcium channels, which ultimately depend on the chloride transport system or by depolarization due to an increase in the external potassium concentrations. It was suspected that this might lead to calcium influx through voltage activated calcium channels [20]. However, nifedipine does not affect calcium channel mediation of initial response to NA. Nifedipine blocks L-type calcium channels activation which is due $GABA_A$ receptor activation-mediated depolarization which may not play a role in the antidepressant action. $GABA_A$ receptor activation increases the release of calcium from the internal stores .Imipramine produces an inhibition of the peak threshold calcium current, which probably decreases the maximum available calcium conductance [14]. It was suggested that imipramine acts by interfering with the influx of extracellular calcium, through both the receptor operated and voltage-gated calcium channels, but does not affect the release of calcium from intracellular storage sites [16, 17].

In the present study I showed that verapamil has an antidepressant-like effect in the rats in the DST model. Treatment with verapamil combined with alprazolam or imipramine produces an additive antidepressant effect, possibly because verapamil has an antidepressant-like effect, but the mechanism is not understood yet. Either imipramine or nifedipine produced a delay in the onset of immobility of 75% and 81%, respectively, compared to the control. Combining nifedipine with imipramine led to a delay of 73% in the onset of immobility compared to the control; which is less than the additive effect. This observation could be explained by the fact that nifedipine has its own antidepressant action mechanism but also blocks the imipramine mechanism that depends on L-type calcium channel activation.

CONCLUSION

Though there was limitation of the study as because it was conducted at only one institution on the limited number of animals, but this study showed that nifedipine possesses antidepressant properties. Combining nifedipine with alprazolam produced an additive antidepressant effect, indicating that different mechanisms were involved. Though there is need of further study in this field to arrive a definitive conclusion.

REFERENCES

1. Frazer A. Pharmacology of antidepressants. J Clin Psychopharmacol, 1997, 17 :(Suppl. 1):2S–18S.
2. Andrews JM and Nemeroff CB. Contemporary management of depression. Am J Med, 1994, 97:24S–32S.
3. Atack JR. Anxioselective compounds acting at the $GABA_A$ receptor benzodiazepine binding site. Curr Drug Targets CNS Neurol Disord, 2003, 2:213–232
4. Roth T, Roehrs TA. Issues in the use of benzodiazepine therapy. J Clin Psychiatry, 1992, 53(Suppl):14–18.
5. Abernethy DR, Schwartz JB. Calcium-antagonist drugs. N Engl J Med, 1999, 341:1447–1457.
6. Alpermann HG, Schacht U, Usinger P, Hock FJ. Pharmacological effects of Hoe 249: A new potential antidepressant. Drug Dev Res. 1992; 25:267–282
7. Nishimura H, Tsuda A, Ida Y, Tanaka M. The modified forced-swim test in rats: Influence of rope- or straw-suspension on climbing behavior. Physiol Behav. 1988; 43:665–668.
8. Porsolt RD, Anton G, Blavet N, Jalfre M. Behavioural despair in rats: a new model sensitive to antidepressive treatments. Eur J Pharmacol. 1978; 47:379–391.\
9. Porsolt RD, Bertin A, Jalfre M. Behavioural despair in mice: A primary screening test for antidepressants. Arch Int Pharmacodyn. 1977; 229:327–336.
10. Porsolt RD, Lenègre A, McArthur RA. Pharmacological models of depression. In: Olivier B, Mos J, Slangen JL (eds) Animal Models in Psychopharmacology, Birkhäuser Verlag Basel. 1991; pp 137–159.
11. Naitoh H, Yamaoka K, Nomura S. Behavioral assessment of antidepressants. The forced swimming test: A review of its theory and practical application. Jpn J Psychopharmacol. 1992; 12:105–111.
12. Nishimura H, Ida Y, Tsuda A, Tanaka M. Opposite effects of diazepam and β-CCE on immobility and straw-climbing behavior of rats in a modified forced-swim test. Pharmacol Biochem Behav. 1989; 33:227–231.
13. Helmeste DM, Tang SW. The role of calcium in the etiology of the affective disorders. Japanese Journal of Pharmacology 1998; 77(2):107-116.
14. Reiser G, Donie F, Binmoller FJ. Serotonin regulates cytosolic Ca2+ activity and membrane potential in a neuronal and in a glial cell line via 5-HT3 and 5-HT2 receptors by different mechanisms. J. Cell Sci. 1989; 93(Pt 3):545-555.
15. Ladewig T, Lalley PM, Keller BU. Serotonergic modulation of intracellular calcium dynamics in neonatal hypoglossal motoneurons from mouse. Brain Res. 2004; 1001(1-2):1-12.
16. Das P, Bell-Horner CL, Huang RQ, Raut A, Gonzales EB, Chen ZL, et al. Inhibition of type A GABA receptors by Ltype calcium channel blockers. Neuroscience 2004; 124(1):195-206.
17. Turovaya AY, Galenko-Yaroshevskii PA, Kade,AKh, Uvarov AE, Kiguradze MI, Khvitiya NG, et al. Effects of verapamil and amiodarone on sympathoadrenal system and balance of excitatory and inhibitory amino acids in rat medulla oblongata. Bull. Exp. Biol. Med. 2005; 139(6):665-667.
18. Sitges M, Reyes A. Effects of verapamil on the release of different neurotransmitters. J. Neurosci. Res. 1995; 40(5):613-621.
19. O'Donnel JM and Shelton RC. Drug therapy of depression & anxiety disorders. In: Brunton LL, Chabner BA,Knollmann BC, editors. Goodman and Gilman's The Pharmacological Basis of Therapeutics.12th ed. New York: McGrawHill; 2011, p 397-415.
20. Vetulani J, Nalepa I. Antidepressants: past, present and future. Eur. J. Pharmacol. 2000; 405:351−363.

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